Development of carotid chemoreceptor dynamic and steady-state sensitivity to CO_2 in the newborn lamb

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1. The maturation of carotid chemoreceptor steady-state and dynamic responses to CO_2 in newborn lambs was measured. In total, sixteen fibres (13 lambs) were studied at 3–4 days, nineteen fibres (13 lambs) at 5–9 days and twenty-one fibres (17 lambs) at 10–24 days after birth.

2. Steady-state CO_2 sensitivity was measured over a range of arterial CO_2 pressures (P_{a,CO_2}) at four levels of arterial O_2 pressure (P_{a,O_2}): hyperoxia (Hyp), 115–150 mmHg; normoxia (Nx), 90–105 mmHg; moderate hypoxia (ModHx), 40–60 mmHg; and severe hypoxia (SvHx), 20–35 mmHg.

3. Steady-state CO_2 sensitivity was present at all ages, and a significant effect of age ($P < 0{\cdot}001$) and P_{a,O_2} ($P < 0{\cdot}025$) (ANOVA) was observed. Older lambs were unable to sustain an increase in chemoreceptor discharge during SvHx as CO_2 was increased.

4. Dynamic CO_2 sensitivity was measured by producing alternations in end-tidal CO_2 levels ($etCO_2$) (alternation amplitude, $1{\cdot}23 \pm 0{\cdot}07\%$ (mean ± S.E.M.); $etCO_2$, $7{\cdot}56 \pm 0{\cdot}15\%$) over 2–8 s at two P_{a,O_2} levels only: 80–100 (Nx) and 40–60 mmHg (ModHx). Peak and trough values of the oscillation in chemoreceptor discharge were plotted against maximum and minimum $etCO_2$ for the control and CO_2-loaded breaths. Dynamic CO_2 sensitivity was calculated as the slope between these points.

5. Dynamic CO_2 sensitivity was greater than steady-state sensitivity in Nx ($P < 0{\cdot}05$) and ModHx ($P < 0{\cdot}01$, Student's paired t test). Unlike steady-state CO_2 sensitivity, there was no significant effect of age or P_{a,O_2} on dynamic sensitivity ($P > 0{\cdot}39$ and $P > 0{\cdot}68$, respectively, ANOVA).

6. Our results show that the neonatal lamb possesses a carotid body steady-state CO_2 sensitivity within a few days of birth, an age when hypoxia sensitivity is low. This CO_2 sensitivity increases with age, perhaps due to the increasing interaction between CO_2 and O_2. Dynamic sensitivity of the carotid body to CO_2 is mature at birth and does not increase with age, as predicted if the response of the carotid body to rapid changes in CO_2 is independent of the sensitivity to the partial pressure of O_2 (P_{O_2}).

Adaptation in the arterial chemoreceptor response to CO_2 has been observed *in vivo* in the adult cat (Leitner & Dejours, 1968; Black, McCloskey & Torrance, 1971) in which a rapid increase in CO_2 produces a brisk increase in discharge frequency followed by a decline to a lower steady level. This adaptation led to the theory that there was both steady-state and dynamic sensitivity of the carotid body to CO_2 (see Torrance, Bartels & McLaren, 1993). Whilst steady-state CO_2 sensitivity is increased at lower levels of arterial O_2 pressure (P_{a,O_2}) levels (Fitzgerald & Parks, 1971), this theory predicts that dynamic CO_2 sensitivity is independent of the level of hypoxia (Goodman, Nail & Torrance, 1974; Band & Wolff, 1978; Kumar, Nye & Torrance, 1988). Chemoreceptor discharge plotted as a function of arterial CO_2 pressure (P_{a,CO_2}) shows that the dynamic response curves are steeper than steady-state response curves, and are parallel at different mean levels of P_{a,CO_2} (Band & Wolff, 1978; Kumar *et al.* 1988). Thus,

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dynamic CO_2 sensitivity of the carotid body in the adult is thought to be independent of the mean level of P_{a,CO_2} and P_{a,O_2}. This is explicable in terms of the bicarbonate hypothesis (Torrance *et al.* 1993), which predicts a brisk response to CO_2 with a relatively greater adaptation to a steady-state level in high oxygen and less in hypoxia.

The neonatal response to hypoxia and the postnatal resetting of hypoxia chemosensitivity has been extensively documented (Blanco, Dawes, Hanson & McCooke, 1984; Kumar & Hanson, 1989; Marchal, Bairam, Haouzi, Crance, Di Giulio, Vert & Lahiri, 1992; Carroll, Bamford & Fitzgerald, 1993). Blanco *et al.* (1984) showed that spontaneous chemoreceptor discharge was present at a P_{a,O_2} of *ca* 25 mmHg in fetal lambs from as early as 90 days gestation, but the chemoreceptor partial pressure of O_2 (P_{O_2}) response curve was displaced to the left of that measured in the adult. Discharge increased when P_{a,O_2} was reduced, when CO_2-equilibrated saline was injected retrogradely into the lingual artery, and when the umbilical cord was occluded. No spontaneous chemoreceptor discharge was recorded on the day of birth in normoxia or hypoxia, however, there was a response to CO_2. Resetting of carotid body sensitivity to hypoxia occurred over the next few days. Marchal *et al.* (1992) measured single fibre chemoreceptor activity in kittens less than and greater than 10 days old at three P_{a,O_2} levels (330, 100 and 50 mmHg), and found that it was less in the younger kittens at P_{a,O_2} 100 and 50 mmHg, but not at 330 mmHg. The P_{O_2} response curve was displaced upwards and to the right in older kittens. Carroll *et al.* (1993) measured whole nerve chemoreceptor activity in kittens aged 1, 4 and 8 weeks and in adult cats at three P_{a,O_2} levels (400, 80 and 40 mmHg). They found that chemoreceptor responses to hypoxia during normocapnia and hypercapnia were greater in 4- and 8-week-old kittens and adult cats compared with 1-week-old kittens. Similarly, a shift of the P_{O_2} response curve upwards and to the right was observed in older kittens.

More recently, postnatal maturation of steady-state CO_2 sensitivity has been reported (Marchal *et al.* 1992; Carroll *et al.* 1993; Pepper, Landauer & Kumar, 1995). Marchal *et al.* (1992) recorded from single chemoreceptor fibres in anaesthetized newborn kittens at two different ages and at three levels of inspired CO_2 in oxygen. Chemoreceptor discharge increased when the partial pressure of CO_2 (P_{CO_2}) increased, and the response of kittens aged 10 days or older was greater than the response for kittens under 10 days old. Carroll *et al.* (1993) measured chemoreceptor responses to CO_2 in anaesthetized kittens aged 1, 4 and 8 weeks and in adult cats at three levels of P_{a,O_2} (40–50, 90–100 and >300 Torr). Chemoreceptor responses to CO_2 were smallest in the youngest kittens at any P_{a,O_2}, and were greater during hypoxia compared with hyperoxia or normoxia, but this interaction between CO_2 and O_2 was only significant at 8 weeks old and in adult cats. Pepper *et al.* (1995) measured single fibre chemoreceptor responses to CO_2 in the rat carotid body *in vitro* in rat pups aged 5–7 days and in adults. CO_2 sensitivity was greater in the adult compared with 5- to 7-day-old pups, and there was no CO_2–O_2 interaction present at the younger age.

In contrast, the dynamic response of the carotid body to CO_2 has been less thoroughly investigated in the neonate. Marchal *et al.* (1992) measured the time taken to reach a steady-state level of chemoreceptor discharge after the application of 5 or 10% CO_2 in oxygen. They found no adaptation, and inferred that the newborn kitten does not possess a dynamic chemosensitivity to CO_2. However, this method does not give a reliable measure of dynamic CO_2 sensitivity, rather it measures the time course of the combined effects of a dynamic response and of adaptation. Other workers have investigated the ventilatory response to inspired CO_2 to measure dynamic CO_2 sensitivity. They reported both an increase (Wolsink, Berkenbosch, DeGoede & Olievier, 1993) and no change (Carroll & Bureau, 1987; Canet, Carroll, Praud, Delacourt & Bureau, 1990) in dynamic CO_2 sensitivity postnatally.

As the observations from ventilatory studies on the chemoreflex response to a rapid change in CO_2 are conflicting, and as there is inconclusive information about dynamic CO_2 sensitivity from chemoreceptor recordings in the neonate, we have measured steady-state and dynamic CO_2 sensitivity from few- and multi-fibre chemoreceptor preparations in anaesthetized newborn lambs. To our knowledge, this is the first study in which chemoreceptor dynamic and steady-state responses to CO_2 have been compared in the neonate.

METHODS

Animal preparation

Lambs were transported on postnatal day 2 or later to holding facilities at University College London, housed in floor pens where ambient temperature was held at 16 °C, humidity at 50% and lighting was fixed on a 12 h light : 12 h dark cycle. They were bottle fed at regular intervals and food was withheld for up to 12 h before an experiment.

Anaesthesia was induced with thiopentone (20 mg kg^{-1} I.V.; Rhône Mérieux Ltd, Essex, UK) and lambs were intubated with a cuffed endotracheal tube (i.d., 4·0–5·5 mm), then artificially ventilated (Sheffield Infant Ventilator Mark 4; Oxford, UK) and anaesthesia was maintained with halothane (1·5–2·5% in O_2). The flow of inspired gases was set using rotameters (Platon, Basingstoke, UK), total flow being 4·0–7·0 l min^{-1}. Lambs were placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA) held in place by ear bars. The trachea was cannulated through a tracheotomy. Gases were sampled from a port in the endotracheal tube and checked using a mass spectrometer (AirSpec 2000; Case Scientific, London, UK). The left femoral vein was catheterized (o.d., 1·34 mm) to allow administration of drugs, and the left brachial artery catheterized (o.d., 1·65 mm) to allow the sampling of preductal arterial blood gases (Instrumentation Laboratory 1306 pH–blood

gas analyser, UK) and the monitoring of arterial blood pressure (ABP, DTX/Plus pressure transducer; Viggo-Spectramed, CA, USA). Blood pressure was maintained if necessary by a 5–10 ml bolus I.V. injection of 10% (w/v) dextran (Sigma) in 0·9% (NaCl) saline as required. Metabolic acidosis was corrected by a 3–5 ml bolus I.V. injection of 8·4% (w/v) $NaHCO_3$ (BDH Chemicals, UK) in 0·9% saline as required. Temperature was maintained at 39·5–40·5 °C by a homeothermic blanket (CFP 8185; BioScience, UK). ECG was recorded using a head stage (NL 100 AK; Digitimer Ltd, UK), amplified and filtered using Neurolog Systems (Digitimer Ltd).

Chloralose anaesthesia (60–70 mg kg^{-1} I.V.; Sigma) was administered and halothane anaesthesia discontinued. Lambs were paralysed with gallamine (5 mg kg^{-1} Flaxedil I.V.; May & Baker, UK). Adequacy of anaesthesia was established by stability of heart rate and arterial blood pressure, and absence of any change in response to pinching either the ear or foot. Once stabilized on chloralose anaesthesia (*ca* 20–30 min), a mid-line incision was made in the neck. The skin was pulled up to form a pool and filled with mineral oil, and all subsequent dissection was performed under mineral oil. The left carotid sinus nerve (CSN) was located and cut at the junction of the glossopharyngeal nerve (see Blanco *et al.* 1984) and the sheath removed. Fine filaments were dissected from the nerve on a blackened plate. Their activity was recorded using stainless-steel hook electrodes, one of which was earthed (NL 100 AK; Digitimer Ltd). Signal conditioning was performed by Neurolog Systems (Digitimer Ltd). The CSN signal was pre-amplified (2000–5000 times), filtered (between 500 and 5000 Hz) and further amplified (100–1000 times).

Carotid chemoreceptor activity was identified by its randomness, lack of synchrony with the ECG and brisk increase in discharge in response to reducing the inspired O_2 fraction (F_{I,O_2}) to nearly zero. Discharge was integrated (Neurolog pulse integrator) and counted in 200 ms bins (Neurolog period generator). Arterial blood pressure (ABP), ECG, raw and integrated chemoreceptor discharge were displayed on an oscilloscope and recorded onto chart paper (Cardioscript CD 6000; Picker, Germany) along with end-tidal oxygen (etO_2) and end-tidal carbon dioxide ($etCO_2$) levels. All signals, including ventilator and gas switching signals, were passed to a pulse code modulator (Sony, Japan) for conversion to video format and then recorded on VHS videotape (Panasonic, Japan).

CO_2 alternations

Ventilation was controlled by a purpose-built ventilator which set respiratory frequency and the ratio of inspiratory-to-expiratory time. It also controlled three solenoids, one that closed the expired line and produced inflation of the lungs, and two (two-way) that allowed inspired gas to be switched between two gas delivery lines. The switching of inspired gas occurred at the start of expiration on a cyclical basis, typically on each breath. When a two-way solenoid was open, gas in that delivery line was inspired by the lamb, and when the solenoid was closed gas was diverted to atmospheric air. Alternations in inspired CO_2 occurred when the two gas delivery lines had different inspired CO_2 fractions (F_{I,CO_2}), typically of low and high CO_2 content. Alternations in $etCO_2$ were then produced on a single-breath basis.

Raw CSN discharge was window discriminated and action potentials were converted to transistor–transistor logic (TTL) pulses and captured digitally by a data acquisition system (NB-MIO-16 input–output board; National Instruments, Austin, TX, USA). LabView software (Apple Computer Inc., Santa Monica, CA, USA) running on a Mackintosh Quadra 630 (Apple Computer Inc.) was used for analysis. Discharge was summed in 200 ms bins over the period of the alternation cycle (i.e. over 2 breaths for a single-breath $etCO_2$ alternation), always starting on inspiration. Summing of discharge could be continued for as long as desired.

Experimental protocol

Steady-state responses. Carotid chemoreceptor dynamic responses to CO_2 were measured in fifty-six fibres from forty-three lambs between 3 and 24 days old. Discharge was measured at several P_{a,CO_2} levels at each of four P_{a,O_2} levels: 115–150 (hyperoxia, Hyp), 90–105 (normoxia, Nx), 40–60 (moderate hypoxia, ModHx) and 20–35 mmHg (severe hypoxia, SvHx).

Dynamic responses. Carotid chemoreceptor dynamic responses to CO_2 were measured in twenty-one of the fifty-six fibres (16 lambs aged between 3 and 17 days) before measuring steady-state chemoreceptor responses at the same P_{a,O_2}. Lambs were ventilated at 0·3–0·5 Hz and inspired gas was switched at the start of expiration on a single breath-by-breath basis between two gas lines (alternation period, 6–8 s). One gas line contained no added CO_2, and to the other 0·4–0·5 l min^{-1} CO_2 was added (total flow 4·0–7·0 l min^{-1}) in Nx (P_{a,O_2}, 80–100 mmHg) and ModHx (P_{a,O_2}, 40–60 mmHg). Arterial blood was sampled during the alternation period to determine P_{a,O_2}.

Data analysis

Values are given as means ± S.E.M. Few- and multi-fibre chemoreceptor preparations were recorded, so it was necessary to normalize discharge to a percentage of maximum to permit comparison between preparations. The maximal response was measured as that to 20–30 s of inspired nitrogen whilst $etCO_2$ was held constant by addition of CO_2 to the inspirate.

Steady-state responses. Chemoreceptor discharge was averaged over 20 s, a minimum of 3 min after a change in $etCO_2$. Linear regression was used to describe the responses of the individual chemoreceptor preparations and the slope of the line taken to indicate CO_2 sensitivity. Two-way ANOVA was used to assess the effect of P_{a,O_2} and age on steady-state CO_2 sensitivity.

Dynamic responses. The voltages applied to the solenoids were used by the computer to detect the start of inspiration, and the start and end of the alternation period. Discharge summation began on inspiration, approximately 3 min after the alternation in $etCO_2$ commenced. The oscillation in CSN discharge was smoothed using a five-point moving average, and the peak and trough of the oscillation was expressed as discharge frequency (in Hz) and corrected for the number of cycles over which summation occurred. Hence, each point of the CSN oscillation was expressed as an average when corrected for the number of alternation periods.

Dynamic CO_2 sensitivity was quite simply measured as the change in discharge frequency produced for a given change in $etCO_2$ when alternating on a single-breath basis. Thus, the slope between the peak and trough in CSN discharge and the corresponding $etCO_2$ were used to derive the dynamic response to CO_2. In this way discharge frequency, normalized to a percentage of maximal discharge, is expressed as a function of $etCO_2$, and dynamic CO_2 sensitivity is calculated from the slope of the oscillation.

Dynamic CO_2 responses were compared with steady-state CO_2 responses for the same chemoreceptor preparation at the appropriate P_{a,O_2} using Student's paired t test. Two-way ANOVA was used to assess the effect of P_{a,O_2} and age on dynamic CO_2 sensitivity.

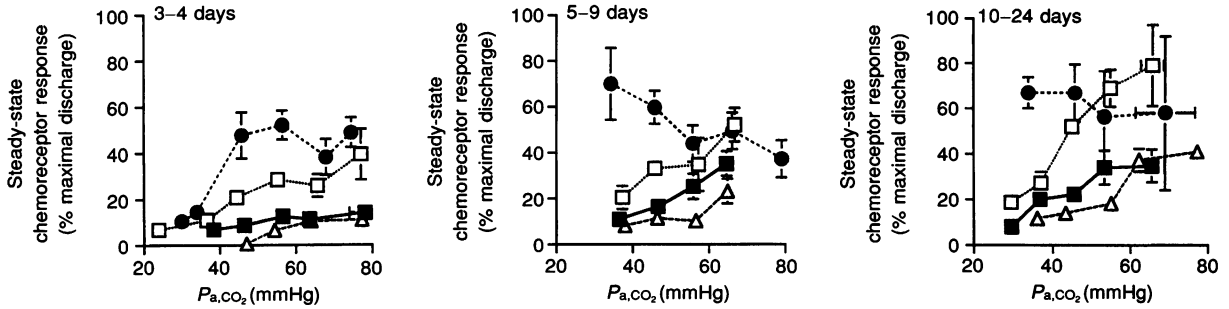

Figure 1. Steady-state chemoreceptor responses to CO_2 plotted as P_{a,CO_2} groups

Chemoreceptor responses shown during Hyp (△), Nx (■), ModHx (□) and SvHx (●); x error bars indicate the S.E.M. for P_{a,CO_2} groups over which responses were averaged; y error bars indicate the S.E.M. for chemoreceptor responses for each P_{a,CO_2} group.

RESULTS

Steady-state chemoreceptor response to CO_2

Steady-state chemoreceptor responses in few- or multi-fibre preparations were recorded in sixteen fibres (13 lambs) at 3–4 days, nineteen fibres (13 lambs) at 5–9 days and twenty-one fibres (17 lambs) at 10–24 days in response to increases in P_{a,CO_2} between *ca* 20 and 80 mmHg at the four levels of P_{a,O_2}. It was not possible to make recordings for all fibres at all P_{a,O_2} levels. During SvHx some lambs were unable to sustain chemoreceptor discharge when P_{a,CO_2} increased. Three of five fibres at 3–4 days, two of three fibres at 5–9 days and three of four fibres at 10–24 days were unable to sustain an increase in discharge frequency as P_{a,CO_2} increased. Figure 1 shows chemoreceptor responses for lambs at three ages during Hyp, Nx, ModHx and SvHx,

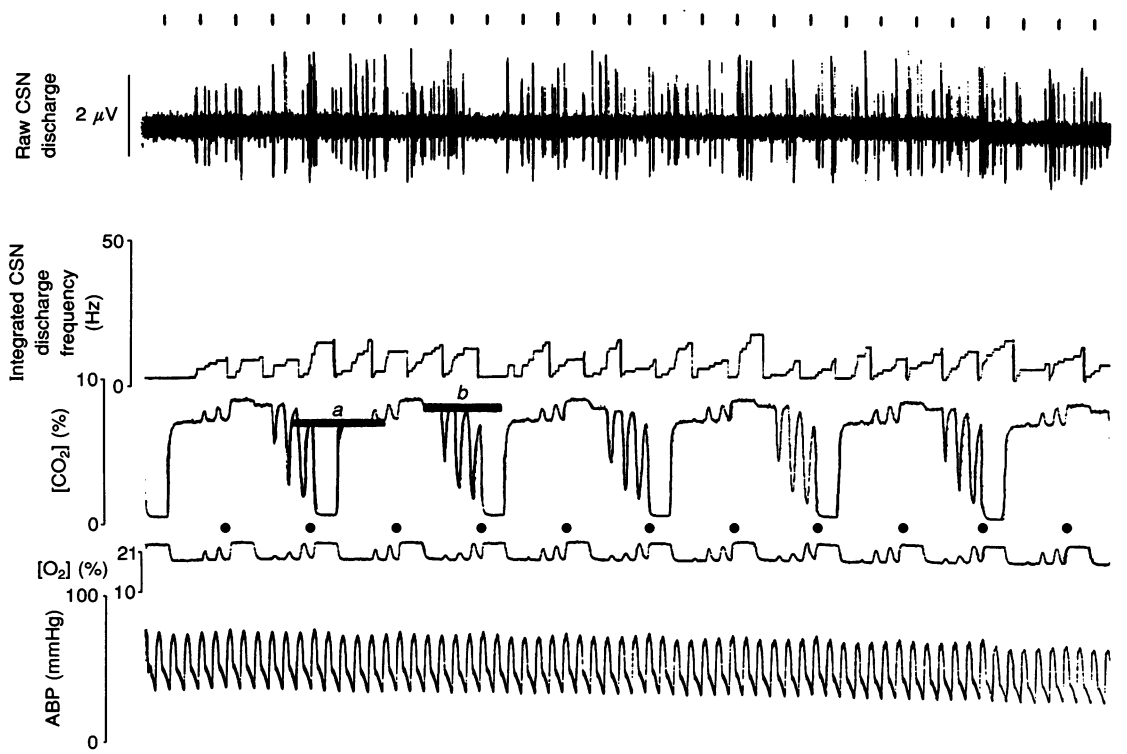

Figure 2. Chemoreceptor discharge during single-breath etCO_2 alternation for a 14-day-old lamb during Nx

Raw and integrated CSN discharge, [CO_2] at the trachea, [O_2] at the trachea and ABP. The ticks at the top of the figure indicate 1 s time intervals. The etCO_2 concentrations for breaths when CO_2 was (*a*; minimum level) and was not (*b*; maximum level) added to the inspirate are indicated, these values are used to calculate dynamic CO_2 sensitivity (see text). Circles between the [CO_2] and [O_2] traces indicate the start of inspiration with low or high CO_2 gas. For [CO_2] and [O_2], the intermediary fluctuations show the sensitivity of the mass spectrometer to cardiac rhythm, and the artefact produced in sampling very small volumes of gas before a high or low concentration is reached.

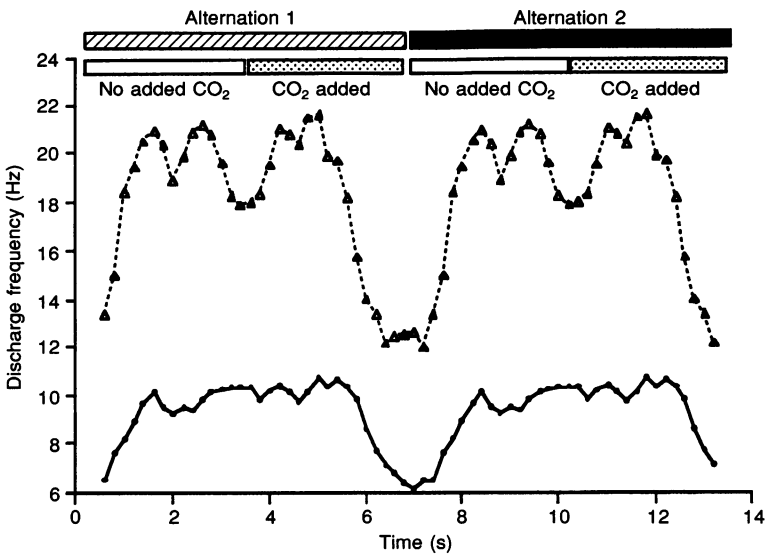

Figure 3. Chemoreceptor discharge during two $etCO_2$ alternations (Nx and ModHx) in a fibre from a 14-day-old lamb

For the purpose of illustration only, the alternation period, corresponding to two breaths (one when no CO_2 was added to the inspirate, followed by another when CO_2 was added to the inspirate), is repeated so that the start and end of the alternation can be seen. The $etCO_2$ alternation during ModHx (upper trace) shows a chemoreceptor oscillation with a greater mean level of discharge than during Nx (lower trace).

grouped at different levels of P_{a,CO_2}. At each age discharge frequency increased when CO_2 increased, except in SvHx when discharge reached a plateau or decreased.

Chemoreceptor responses during SvHx were therefore excluded from analysis to assess the effect of age and P_{a,O_2}. A significant effect of age and P_{a,O_2} on CO_2 sensitivity was observed ($P < 0{\cdot}004$ and $P < 0{\cdot}01$). There was no significant interaction between age and P_{a,O_2} ($P > 0{\cdot}8$).

Dynamic chemoreceptor response to CO_2

For lambs aged 3–4 days, two fibres in Nx and five fibres in ModHx were recorded, at 5–9 days five fibres in Nx and five fibres in ModHx were recorded, and at 10–17 days seven fibres in Nx and five fibres in ModHx were recorded. Figure 2 shows an example of a single breath alternation in $etCO_2$ for a lamb aged 14 days during Nx; *a* and *b* indicate the $etCO_2$ levels used to measure CO_2 sensitivity. The mean amplitude of the alternation in $etCO_2$ for all responses was $1{\cdot}23 \pm 0{\cdot}07\%$ about a mean level of $7{\cdot}56 \pm 0{\cdot}15\%$. Figure 3 shows an oscillation in discharge for a fibre at 14 days during Nx and ModHx. The mean level of discharge is increased in ModHx, and the amplitude is also increased in this fibre. There is a delay in response time between an increase in $etCO_2$ and an increase in chemoreceptor discharge of *ca* 3–4 s.

Figure 4 shows an example of dynamic chemoreceptor responses during Nx and ModHx, and their corresponding steady-state responses for one fibre from a 6-day-old lamb. Steady-state CO_2 sensitivity was greater in ModHx compared with Nx, and dynamic chemoreceptor responses were greater than steady-state chemoreceptor responses. Dynamic CO_2

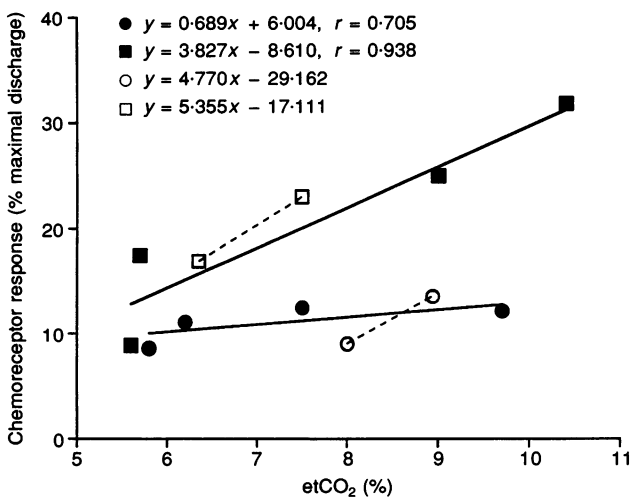

Figure 4. CO_2 sensitivities during Nx and ModHx for a fibre from a 6-day-old lamb

Steady-state (filled symbols) and dynamic (open symbols) CO_2 sensitivities during Nx (circles) and ModHx (squares) for one fibre from a 6-day-old lamb. Chemoreceptor response is plotted as a function of $etCO_2$. Linear regression describes the slope (CO_2 sensitivities) of the responses.

Table 1. Dynamic chemoreceptor responses to single-breath alterations in $etCO_2$ during Nx and ModHx

Age group (days)	Chemoreceptor response	
	Nx	ModHx
3–4	4·28 ± 0·07	9·47 ± 2·27
5–9	9·11 ± 2·62	6·07 ± 1·19
10–17	5·53 ± 1·12	11·71 ± 1·79

Dynamic chemoreceptor response is given as the slope of the response curve (% maximal discharge/% $etCO_2$).

chemoreceptor responses for each fibre were compared with their steady-state responses, and were significantly greater during both Nx ($P < 0·05$) and ModHx ($P < 0·01$). Mean dynamic CO_2 sensitivities for each age group are summarized in Table 1. There was no significant effect of age ($P > 0·39$) or P_{a,O_2} ($P > 0·68$) on dynamic CO_2 sensitivity.

DISCUSSION

We have found a steady-state CO_2 sensitivity in lambs aged 3–24 days that increases both with age and at lower levels of P_{a,O_2}. This confirms the observations of Marchal *et al.* (1992) and Carroll *et al.* (1993) in the kitten *in vivo*, and agrees with the observations of Pepper *et al.* (1995) in the rat *in vitro*. We observed that older lambs were unable to sustain an increase in chemoreceptor discharge during SvHx when CO_2 increased, whereas in younger lambs discharge reached a plateau. We have demonstrated the presence of dynamic CO_2 sensitivity in the neonate using a method that alternates $etCO_2$ on a single-breath basis. Dynamic CO_2 sensitivity was present in all lambs between 3 and 17 days old and it was independent of age and P_{a,O_2}.

We found that it was only possible to dissect to the level of few- or multi-chemoreceptor fibres which necessitated normalization of the data. We chose to normalize chemoreceptor discharge to maximal discharge, in preference to a normocapnic normoxic level, because it was difficult to set the criteria for the latter. If a normocapnic normoxic level was chosen on the basis of blood gas analysis, then a P_{a,CO_2} of 40 mmHg and P_{a,O_2} of 100 mmHg may be normocapnic and normoxic for a lamb aged 12 days, but not for one aged 3 days. It was therefore more appropriate to normalize results to maximal discharge.

It is likely that our recordings were representative of myelinated fibres as these would show the largest action potentials which were favoured in the interest of a good signal-to-noise ratio. However, we were not able to measure the conduction velocities of these fibres to determine whether they were in fact myelinated. We chose not to section the efferent sympathetic innervation of the carotid body from the superior cervical ganglion, namely the ganglioglomerular nerves, as this innervation may modify chemoreflexes, and we wanted our results to be interpretable in the context of the whole animal. However, there is evidence that sympathetic innervation in fact has a negligible role in determining carotid body steady-state sensitivity to hypoxia and CO_2 (Davies, Nishino & Lahiri, 1981; McQueen, Evrard, Gordon & Campbell, 1989). In addition, our steady-state responses are similar to those of Carroll *et al.* (1993) who sectioned the ganglioglomerular nerves in newborn kittens. Thus, it is unlikely that there was a significant effect of carotid body sympathetic innervation on chemoreceptor responses.

We found that there was a significant effect of P_{a,O_2} on carotid body steady-state CO_2 sensitivity during Hyp, Nx and ModHx, so that at lower oxygen levels CO_2 sensitivity was increased. Carroll *et al.* (1993) found that chemoreceptor responses to CO_2 were increased at lower oxygen levels in kittens aged 1 and 4 weeks, but that significant CO_2–O_2 interaction was only present at 8 weeks and in adult cats. More recently, Pepper *et al.* (1995) have postulated that the postnatal development of carotid body hypoxia sensitivity is due to the emergence of significant interaction between O_2 and CO_2. The chemoreceptor response to CO_2 was increased during hypoxia by an upward shift in chemoreceptor discharge at 5–7 days, but CO_2 sensitivity was unchanged. Adult rats showed a multiplicative effect between CO_2 and O_2. However, the observations of Pepper *et al.* (1995) do not address the time course of the development of interaction, as only one age group of neonates was compared to the adult.

Chemoreceptor responses to CO_2 during SvHx (P_{a,O_2} 20–35 mmHg) differed from responses obtained during Hyp, Nx and ModHx. Lambs aged 3–4 days showed an initial increase in discharge which reached a plateau, and lambs aged 5–9 days and 10–24 days showed a decrease in discharge as P_{a,CO_2} increased. To our knowledge, this effect of CO_2 at low O_2 has not been reported in the neonate, however, some workers report that chemoreceptor discharge is not maintained during sustained isocapnic hypoxia (Kumar & Hanson, 1989; Mulligan & Bhide, 1989; Marchal *et al.* 1992; Carroll *et al.* 1993). In the adult, Hornbein, Griffo & Roos (1961) reported an effect of CO_2 on chemoreceptor discharge at low P_{a,O_2} levels. They observed at $P_{a,O_2} < 35$ mmHg during hypocapnia, and $P_{a,O_2} < 25$ mmHg during normocapnia, that chemoreceptor discharge was less compared with higher P_{a,O_2} levels in adult cats. Kumar & Hanson (1989) found in aortic chemoreceptor fibres that lambs aged 0–19 days ($n = 15$) were unable to sustain discharge below 30 mmHg, whilst lambs aged 1–4 days ($n = 15$) were able to show an increase in chemoreceptor discharge for reductions in P_{a,O_2} to *ca* 25 mmHg. Kholwadwala & Donnelly (1992) found in rat carotid body *in vitro* that during prolonged periods of anoxia and severe hypoxia, the reduction from peak chemoreceptor discharge was greater in adults than pups aged 1–2 days. The mechanism for the decrease in discharge seen in older

animals during severe hypoxia or asphyxia is unknown, however, it is likely to be linked to hypoxic resetting of the carotid body from the fetal range. It suggests that chemoreceptor discharge 'fails' in older lambs, and is not due to chemoreceptor adaptation. Adaptation implies that a maximal response is reached after which time discharge decreases to a lower level, however, this effect was observed primarily in older lambs and not in younger ones.

Despite the lack of maintained discharge in SvHx as CO_2 increased, our observations are not easily explained by those of other workers, who report that chemoreceptor discharge is not maintained during periods of relatively moderate hypoxia. Carroll *et al.* (1993) found that in four of six kittens aged 1 week, and one of six kittens aged 4 weeks, the chemoreceptor response to hypoxia (P_{a,O_2}, 40–45 mmHg) was 'biphasic', reaching a peak within the first 30 s, and then adapting to a lower level by 2 min. Marchal *et al.* (1992) also reported a biphasic chemoreceptor response to hypoxia (P_{a,O_2}, 55 mmHg) in eight of fifteen kittens aged less than 10 days, and three of nine kittens aged greater than 10 days. They found that peak discharge was reached after 20 s, was maintained for *ca* 15 s and then gradually declined to a lower steady-state level after another 15 s. Our observations were all made more than 3 min after changing the stimulus levels, and it is therefore unlikely that an adaptive response to hypoxia accounts for our observations. Chemoreceptor failure at very low P_{a,O_2} levels is a more likely explanation for our findings in older lambs, but it is not clear why this is age dependent.

We maintain that this is the first study to measure dynamic chemoreceptor response to CO_2 in the neonate. Marchal *et al.* (1992) previously reported an absence of dynamic sensitivity to CO_2 in the newborn kitten because they did not observe an overshoot in the chemoreceptor response to inhaled 5 or 10% CO_2. They compare their results to those of Black *et al.* (1971), yet their methodology is fundamentally different because they did not produce a sufficiently rapid stimulus at the carotid body to produce an overshoot in discharge. In addition, discharge was analysed in 10 s periods until a steady state was reached, which is too slow to measure dynamic sensitivity. To base conclusions on the presence or absence of dynamic sensitivity solely on the speed of response to inhaled CO_2 in this way is incorrect. We have provided firm evidence for carotid chemoreceptor dynamic CO_2 sensitivity in the neonate and found no effect of age on this sensitivity.

The findings from ventilatory studies in which the dynamic component of the respiratory chemoreflex to CO_2 was measured are conflicting. Carroll & Bureau (1987) and Canet *et al.* (1990) both reported no increase in dynamic CO_2 sensitivity postnatally. Wolsink *et al.* (1993) and Upton, Milner, Stokes & Wilson (1990) found a maturation of the dynamic ventilatory response to CO_2, however, it is difficult to exclude the involvement of the central chemoreceptors when respiration is measured. In addition postnatal changes in lung compliance have to be borne in mind. LaFramboise, Tuck, Woodrum & Guthrie (1984) and LaFramboise, Guthrie, Standaert & Woodrum (1983) showed in newborn monkeys that the increase in dynamic lung compliance between 2 and 21 days counted for 38% of the postnatal increase in minute ventilation. Thus, direct recording of chemoreceptor afferent activity is the only accurate method to determine dynamic arterial chemoreceptor CO_2 sensitivity.

We found no significant effect of P_{a,O_2} on the dynamic chemoreceptor response to single breath alternations in $etCO_2$, which is expected from reports in the adult cat. The amplitude of the oscillation in chemoreceptor discharge during the steady state can also be taken as an index of dynamic CO_2 sensitivity, and Goodman *et al.* (1974) and Band & Wolff (1978) found that it was unchanged during hypoxia. Kumar *et al.* (1988) found using a high-frequency high-flow ventilator that the amplitude and shape of the chemoreceptor oscillation was not significantly altered by a change in P_{O_2}. They also showed that the CO_2 transient response curves were parallel at different P_{O_2} levels and mean P_{CO_2} levels, as described by Torrance *et al.* (1993). Thus, for an increase in arterial P_{CO_2}, the carotid chemoreceptors respond along one of the parallel transient response curves and then adapt down to a steady-state discharge level dependent on the P_{O_2}. Because the slope of the transient CO_2 response curve is always the same, during hypoxia the chemoreceptors adapt little and during hyperoxia they adapt considerably. Our results suggest that dynamic CO_2 sensitivity is P_{O_2} independent in the neonatal lamb, and hence in this respect it is similar to adult chemoreceptor responses.

Our finding that there is a carotid body dynamic CO_2 sensitivity present at birth which does not increase with age, unlike hypoxia sensitivity and steady-state CO_2 sensitivity of the carotid body, is important for respiratory control in the newborn, because until hypoxia sensitivity has reset the response to CO_2-induced oscillations in chemoreceptor discharge may be the most important stimulus for ventilatory control. Changes in P_{a,CO_2} provide an important stimulus for the initiation of continuous breathing at birth (Woodrum, Parer, Wennberg & Hodson, 1972; Kuipers, Maertzdorf, de Jong, Hanson & Blanco, 1994). The amplitude of the oscillation in chemoreceptor discharge is determined by the P_{a,CO_2} oscillations, and is influenced by metabolism (Cross *et al.* 1982). This relationship between metabolism and ventilation, and the role of CO_2 in controlling ventilation, has important implications for the role of peripheral chemoreceptor function in the aetiology of sudden infant death syndrome (SIDS). Both hyperthermia and infection have been suggested as contributing factors for SIDS (Wigfield, Gilbert & Fleming, 1994; North, Petersen & Wailoo, 1995). If a poor respiratory chemoreflex response to CO_2 contributes to respiratory failure in SIDS, the problem could lie at the level of the carotid chemoreceptor in detecting oscillations in arterial CO_2, in the transmission of the afferent signal to the brainstem, or in the ability of the respiratory controller to initiate an appropriate ventilatory response. Our results

provide information on the normal development of the afferent limb, i.e. the arterial chemoreceptor responses to CO_2, against which future work on the effect of pathological conditions can be assessed.

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